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CLINICAL AND IMMUNOLOGIC RESPONSES IN PATIENTS WITH VIRAL KERATOCONJUNCTIVITIS

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Ocular viral infections manifest themselves in a variety of clinical pictures. Most commonly, the clinician finds follicular conjunctivitis, with or without accompanying keratitis. Epithelial and subepithelial corneal infiltrates are frequently observed with adenovirus infections, but also occur with herpes simplex and other viral-induced keratitis infections.^{1,2} In addition to keratoconjunctivitis, viruses can (directly or indirectly) cause deep ocular involvement, including uveitis and optic nerve inflammation.¹

In an effort to learn more about the etiologies of ocular viral infections and the pathogenesis of viral keratitis, we studied two groups of patients with epidemic and non-epidemic viral infections. All of them had follicular conjunctivitis, and many suffered from keratitis during at least one stage of their illness.

We had three major goals: to determine the rate of viral recovery from cases of non-epidemic acute or subacute follicular conjunctivitis; to compare the accuracy of clinical diagnoses with that achieved by culture or serologic studies, or both; and to explore the possible role of serum antibody in the formation and maintenance of corneal subepithelial infiltrates.

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We examined one group of patients who were involved in an epidemic caused by a single viral agent, and a second group of patients who were infected with many different viruses. Our results indicate that a variety of agents can cause similar illness patterns, and that clinical observation cannot always distinguish one from another.

Furthermore, serologic tests are a more accurate diagnostic method than clinical course in ocular viral illness, as is the case in systemic viral illnesses. Finally, viral antibody titers may correlate directly with specific clinical changes in adenovirus-induced keratoconjunctivitis.

MATERIAL AND METHODS

Patients—We studied two groups of patients by the following methods. The first was a population of 56 industrial employees who had epidemic keratoconjunctivitis. These patients were described in detail elsewhere,³ but the data concerning their ocular findings were not presented.

The second group of 64 patients comprised a heterogeneous population seen during an 18-month period. The only criterion for selection was follicular conjunctivitis. All but nine patients were seen in the emergency room, clinic, or staff offices at Barnes Hospital from November 1971 to December 1972. The other nine were followed at the National Eye Institute from July 1970 to June 1971. One of us (H.L.S.K.) saw every patient during the acute or subacute phase of the illness and followed them until signs and symptoms abated. There were 32 males and 32 females, ranging in age from 3 to 77 years old (mean, 31.8 years). Twenty-nine

TABLE 1
ORGANISMS TESTED FOR IN THE SEROLOGIC STUDY

Influenza A, B, and C
Parainfluenza 1, 2, 3, 4A, and 4B
Newcastle disease virus
Mumps
Rubeola
Respiratory syncytial virus
Coronavirus OC-43, 229E
Vaccinia
Herpes
Varicella
Cytomegalovirus
Adenovirus types 1-33
Coxsackie A 7, 9, 16, and 21
Coxsackie B 1, 2, 3, 4, 5, and 6
Echovirus 4, 28
Acute hemorrhagic conjunctivitis
<i>Mycoplasma pneumoniae</i>
<i>Bedsonia</i> (TRIC)

patients were black, 33 were white, and two were orientals. All were ambulatory and in good health.

Collection of virus culture specimens—Fifty-eight of the 64 nonepidemic patients had viral cultures. A cotton swab was rolled in the inferior cul-de-sac and transferred to a carrier medium (sodium chloride, potassium chloride, dipotassium phosphate, charcoal, and agar, pH 7.0). The contents of this tube were either stored at -70°C for a short period or immediately transferred to tissue culture tubes of human diploid fibroblast, primary human embryo kidney cell, or human epidermoid carcinoma. Standard culture methods have been described previously.⁴ All viruses isolated in this initial attempt were passed and compared to reference prototype virus strains for indication of atypicality.

The methods for viral culture in the 56 epidemic keratoconjunctivitis patients have been described elsewhere.³ A single patient was cultured for trachoma inclusion conjunctivitis (TRIC) agent by other methods.⁵

Serologic studies—Of the original 64 study patients, 13 did not return for a convalescent serum. Therefore these serologic results are based on 51 patients. We collected sera dur-

ing the acute phase and once or twice during the convalescent phase. There were two family groups in which a specimen from one uninfected member was considered acute and another infected member's specimen was considered convalescent. We took a convalescent specimen from 34 of the 51 patients within 30 days of the initial visit; 14 within 60 days; and three patients had specimens that were separated by more than 60 days.

The sera were tested for antibodies to the prototype strains of many respiratory and other viruses in order to survey all of the likely causes of follicular conjunctivitis (Table 1). Complement-fixation, hemagglutination-inhibition, indirect hemagglutination, or serum neutralization tests were employed as appropriate for each particular virus. The sera were heat-inactivated (56°C , 30 minutes) for all serologic tests. Antibodies to influenza A, B, and C, parainfluenza 1, 2, and 3, mumps virus "viral antigen," rubeola, respiratory syncytial virus (RS v), coronavirus OC-43 and 229E, herpes simplex 1, varicella, *Mycoplasma pneumoniae*, *Bedsonia* (TRIC agent), cytomegalovirus, and adenovirus group-specific hexon-antigen were assayed by the standardized complement-fixation test with overnight fixation of 5 units of complement.^{4,6} Antibodies to influenza A and B, parainfluenza 1, 2, 3, 4A, and 4B, Newcastle disease virus, mumps virus, rubeola, coronavirus OC-43, vaccinia, and adenovirus types 1 to 33 were assayed by the standardized hemagglutination-inhibition test with 0.01M phosphate-buffered saline diluent and spectrophotometrically standardized 0.4% mammalian or 0.5% avian red blood cells.⁷ Antibodies to coronavirus 229E were additionally measured by an indirect hemagglutination test.⁸ Neutralizing antibodies to rubeola, RS v, selected adenoviruses, the six coxsackie B viruses, echovirus types 4 and 9, and coxsackievirus A-10 were assayed by serum neutralization tests in human epidermoid carcinoma or monkey kidney tissue culture.⁹ Neutralizing

antibody titers to vaccinia, herpes, coxsackievirus A-9, A-16, and A-21, and four strains of acute hemorrhagic conjunctivitis virus were similarly assayed by serum neutralization tests in human diploid fibroblast tissue culture. Serum neutralization antibodies in coxsackievirus A-4 and A-7 were assayed in suckling mouse brain.¹⁰

For diagnostic purposes a fourfold titer change (either rise or fall) was considered significant for any of the serologic tests. In addition a stable titer of $\geq 1:32$ in any serologic test (hemagglutination-inhibition, serum neutralization, or complement-fixation) suggested infection with that agent if the clinical course and other serologic tests were confirmatory.

When serologic comparisons were carried out, as with the adenovirus groups described below, all of the testing was done in a standardized manner. We used only the hemagglutination-inhibition test, with 0.01M phosphate-buffered saline and standardized mammalian or avian red blood cells, to compare adenovirus types. Antigens for types 1 to 33 were maintained at the Center for Disease Control and were used for similar serologic studies in the past.⁷ Other serologic test results did not enter into the calculations for significance of titer levels. We tested all sera simultaneously and in duplicate with the same cell lots whenever possible so that testing conditions and results would be equivalent.

Clinical examinations—Those patients who had epidemic keratoconjunctivitis⁸ were examined during the initial phase of their illness and approximately one month after resolution of all symptoms. Visual acuity was recorded and, by slit-lamp microscopy, we made a determination of presence or absence of subepithelial infiltrates. There was no attempt to quantitate these or to judge severity.

The patients in the nonepidemic group were treated in a different manner. All patients were seen at least twice for evaluation of subjective and objective evidence of ocu-

lar illness. During each examination, we attempted to document severity of symptoms, visual acuity, the presence of pre-auricular nodes (or other adenopathy), and the appearance of the conjunctiva, cornea, and anterior chamber by slit-lamp microscopy. Detailed drawings of the cornea were made in keratitis cases, but the exact number of lesions was not recorded. Instead, we attempted to depict accurately the areas of cornea involved for future evaluation. Each patient was followed until keratitis showed signs of stability or actual regression. When possible, those patients with persistent lesions were followed for a longer time period.

At the end of the study period, all of the drawings were re-examined. For each patient we estimated the percentage of cornea involved with infiltrates during the peak phase of the illness. We assigned values before the serologic data were available. When all of the serologies were complete, the adenovirus groups were investigated separately. We calculated the severity of keratitis vs. serologic titer by hemagglutination-inhibition test values and the percentage of corneal involvement arrived at previously.

Therapeutic regimen—Almost all of the patients were treated with topical antibiotics, compresses, or astringents. Two of the patients in the adenovirus group received corticosteroids after cessation of the acute phase and when visual acuity decreased. Patients in the herpes group were given various antiviral preparations. Although these were generally effective in controlling the acute disease, we did not attempt statistical correlations to determine efficacy.

Ancillary studies—We took swabs for routine bacterial cultures in 26 nonepidemic patients simultaneously with the viral cultures. Although most of these were taken at random, occasionally patients presented with signs and symptoms suggestive of bacterial conjunctivitis complicating the viral illness; these patients were cultured specifically to identify possible infectious agents. In addition, conjunctival scrapings were examined

TABLE 2
RELATIONSHIP OF LENGTH OF ILLNESS
TO VIRAL RECOVERY

	≤7 Previous Symptom Days	≥8 Previous Symptom Days
Adenovirus 3	2/4*	0/1
Adenovirus 7	3/6	0/6
Adenovirus 8	7/9	1/4
Herpes simplex	2/6	0/3
Herpes zoster	1/1†	0/2
Other‡	0/10	0/6
Total	15/36§	1/22

* Number of positive isolates per number of patients cultured.

† Skin lesion was positive, eye culture negative.

‡ Includes individuals who had cultures but no serologic studies; an additional patient (not included) had an isolate of adenovirus type 16, but she was unsure of the duration of her illness.

§ $\chi^2 = 9.5$; $P < .005$.

in 27 patients. These were processed with Giemsa or Gram stains, or both.

Tear studies—Tears were collected from ten patients with acute and chronic, nonepidemic viral infections or chronic noninfectious conjunctivitis. The details for collecting, processing, and quantitating immunoglobulins in tears have been described previously.^{11,12} The viral serologic studies on the tear specimens were similar to those done on the sera from these individuals, but the tear specimens were not heat-inactivated and were diluted starting at 1:2 instead of 1:8. (Other studies involving the epidemic keratoconjunctivitis group have been described previously.⁸)

RESULTS

Viral studies (nonepidemic patients)—Although more than 90% of the infected eyes were cultured, the yield of positive viral cultures was low. Table 2* shows the difference between the number of positive isolates obtained by culturing before or after the eighth

day of illness. Culturable virus tended to disappear after the first week of illness in most of the virus groups examined.

Adenovirus isolates (types 3, 7a, 8, and 16) were typical prototype-like strains but three of the adenovirus type 7a viruses were not; these were neutralized or inhibited less well with prototype reference antisera than with the patient's own convalescent serum.¹³ This was not clinically significant.

Serologic studies (nonepidemic patients)—Since positive viral cultures were sparse, the retrospective study of acute and convalescent sera constituted the major means of diagnosis. If cultures were positive or if clinical evidence pointed to a specific diagnosis—the presence of herpetic dendrites—the total battery of serologic tests (Table 1) was not always carried out.

We compared the clinical and serologic diagnoses in the 51 patients whose sera were examined (Table 3). All patients with positive cultures showed the expected serologic changes. Two patients appeared to have experienced infections with two agents, either simultaneously or in close temporal proximity. In one patient, the hemagglutination-inhibition titer to adenovirus type 7 was stable at 1:128, indicating a recent infection; at the same time, the culture for TRIC agent was positive.⁵ The second patient had an illness characterized by initial upper respiratory infection and secondary chronic unioocular keratitis, with corneal ulceration and recurrences. Over the three months of observation during the chronic phase, he developed a gradual fourfold rise in adenovirus type 7 hemagglutination-inhibition antibody (1:32 to 1:128). Almost eight months after his initial illness he demonstrated signs of anterior uveitis after an upper respiratory illness (nonspecific) and increased his herpes simplex antibody titer fourfold (1:32 to 1:128) in both complement-fixation and serum neutralization tests. These data support a premise of double infections in both patients.

Five additional patients demonstrated sero-

* All of the data for the epidemic keratoconjunctivitis group were published elsewhere, except for the corneal quantitative material in clinical studies.⁸

logic changes suggestive of either sequential or simultaneous infection with two or three agents. However, in these patients we could not differentiate clinically among the various agents. Therefore, the serology that best fitted the clinical picture and time sequence of infection was chosen as the final diagnosis.

Most of the clinical misdiagnoses were made in the cases of presumed epidemic keratoconjunctivitis. Adenovirus type 7 was the most prominent masquerader, and herpes simplex passed for epidemic keratoconjunctivitis in one case.

We listed the geometric mean titers for the major adenovirus groups encountered in this study in Table 4. Although the average fold-rise in geometric mean titers was similar, the final absolute titers were different by more than twofold. This difference was statistically significant.

In an attempt to explain the higher final titer of the Group 1 adenovirus-infected pa-

tients, we looked at two different factors. The first was a consideration of a possible difference in the acute and convalescent phase serum samples (Table 5). The two groups were similar in makeup in that nine of 16 Group 1 patients and ten of 13 Group 2 were examined during the acute phase of their illness: they showed a fourfold rise in their titer to the infecting adenovirus.

There was a moderate disparity between patient history and the serologic data. Three Group 1 patients (Cases 8, 9, and 10) claimed they were ill for a few days, but their titer reflected a long-term illness (≥ 32 initial or no change with time, or both). We observed one patient (Case 8) with an unchanging 1:16 titer throughout his illness, and we could not explain the lack of change in titer. This disparity was less evident in Group 2 (Case 26).

The difference in acute phase geometric titer for each group was nearly significant

TABLE 3

CLINICAL DIAGNOSIS COMPARED TO SEROLOGIC OR CULTURE-PROVEN DIAGNOSIS OF VIRAL INFECTIONS

Culture or Serologic Diagnosis	Clinical Diagnosis					
	Epidemic Kerato- conjunctivitis	Pharyngo- conjunctival Fever	Epidemic Kerato- conjunctivitis or Pharyngoconjunc- tival Fever	Herpes Simplex	Herpes Zoster	Follicular Conjunctivitis
Adenovirus Group 1						
Type 3	1	2	1	0	0	1
Type 7	4*	4	2	2	0	3†
Type 16	0	0	1	0	0	0
Group 2						
Type 8	12	0	1	0	0	0
Type 9	0	0	0	0	0	1
Type 29	1	0	0	0	0	0
Group 3						
Type 1	0	0	0	0	0	1
Herpes virus‡	2*	0	0	6	4	3
TRIC agent	0	0	0	0	0	1†
Total	20	6	5	8	4	10

* One patient who had serologic evidence of infection with both herpes simplex virus and adenovirus 7 is included twice.

† One patient who had serologic evidence of infection with adenovirus 7 and a simultaneously positive culture for TRIC agent is included twice.⁵

‡ Herpes group includes herpes simplex, varicella zoster, and cytomegalovirus.

TABLE 4
SEROLOGIC DATA FROM GROUP* AND
TYPES OF ADENOVIRUSES

Adenovirus Type	GMT†	GMT of Sera With ≥ Fourfold Changes	
		Acute	Convalescent
<hr/>			
	Group 1		
	<hr/>		
3	73.5		
7	93.4		
3, 7, 16	84.5‡	9.3	86.3§
<hr/>			
	Group 2		
	<hr/>		
8	41.5		
8, 9, 29	41.8‡	5.6	42.2§

* Grouping based on agglutination studies.^{4,18}

† Geometric mean titer of total group (compare with Table 5).

‡ $t = 2.87$; $P < .01$.

§ $t = 2.64$; $df, 17$; $P < .02$.

by t -test (t) (Group 1 = 21.6, Group 2 = 8.9; $t = 2.00$, 27 degrees of freedom [df]; $.05 < P < .06$). However, when we considered only patients proven to be in the acute phase, the difference was not significant (5.6 vs. 9.3; $P > .1$).

There was a difference in the number of days between acute and convalescent samples for the two groups. Overall, the mean number of days was 21 for Group 1 and 29 for Group 2 (not significant). However, if only the 19 patients in the acute phase were again considered, the averages were 19 days and 32 days, respectively ($P < .05$; $t = 2.34$; $df, 17$). (From these data, a higher titer should have been expected for Group 2 because of the longer interval between acute phase and serum peak.) Thus, neither the titer of acute phase sera nor the sampling time of convalescent phase sera seemed to account for the difference in final antibody levels.

We also considered the degree of upper respiratory or generalized illness among patients in both groups. Of the 21 patients with serologic evidence of Group 1 infection,

only 16 gave accurate histories; nine of these reported an associated upper respiratory infection with conjunctivitis. In contrast, one (with adenovirus type 9) of the 13 patients in Group 2 exhibited signs or symptoms of upper respiratory infection. This difference was again significant ($P < .01$, χ^2 test).

Thus, those with Group 1 nonepidemic adenovirus infection were afflicted with more upper respiratory infections, but these in-

TABLE 5
SEROLOGIC TITER CHANGES DURING ACUTE AND
CONVALESCENT STAGES OF NONEPIDEMIC
ADENOVIRUS KERATOCONJUNCTIVITIS

Case No.	Acute Phase Titer*	Convalescent Phase Titer*	Days Ill on First Visit	No. of Days Between Acute and Convalescent Sera
Group 1				
1	4	128	≤ 7	22
2	4	128	≤ 7	30
3	4	32	≤ 7	12
4	4	128	≤ 7	11
5	8	64	≤ 7	13
6	32	128	≤ 7	40
7	32	128	≤ 7	18
8	16	16	≤ 7	28
9	64	64	≤ 7	14
10	128	128	≤ 7	14
11	8	32	≥ 14	15
12	32	128	≥ 14	18
13	32	64	≥ 14	50
14	64	128	≥ 14	25
15	256	256	≥ 14	12
16	64	64	≥ 14	15
Group 2				
17	8	32	≤ 7	30
18	4	32	≤ 7	50
19	4	32	≤ 7	11
20	4	16	≤ 7	35
21	8	32	≤ 7	21
22	4	32	≤ 7	28
23	4	128	≤ 7	21
24	8	64	≤ 7	45
25	16	64	≤ 7	50
26	32	32	≤ 7	32
27	4	64	11	32
28	64	64	≥ 14	14
29	32	32	≥ 14	14

* Represents reciprocal of highest endpoint dilution in hemagglutination-inhibition test.

fections were not necessarily responsible for the significantly higher serologic titers. In fact, there was no significant relationship between the presence of an upper respiratory infection and a serologic response that equaled or exceeded 1:128 (Table 6). This negative correlation held true for changes in both homologous and heterologous virus titers. Therefore, the explanation for the higher serum titers in patients with Group 1 infections may not be related directly to the concomitant upper respiratory infections.

We compared the sensitivity of the two most commonly employed serologic tests (Table 7). Hemagglutination-inhibition tests demonstrated fourfold rises in many cases where complement-fixation tests failed to show significant changes. Hence, primary dependence on the usual complement-fixation hexon-antigen test would have resulted in fewer positive diagnoses, even in acute infections.

Ancillary laboratory studies (nonepidemic patients)—We microscopically examined conjunctival smears from 21 patients, and routine bacterial cultures were taken from 11 of these patients plus ten other individuals. Cultures from five patients showed coagulase-positive staphylococci; these patients had various diagnoses: adenovirus type 8 epidemic keratoconjunctivitis, adenovirus type 7 pharyngoconjunctival fever, herpes simplex keratitis, and a case of chronic "facitious" conjunctivitis.

The smears were helpful only in cases of herpetic infections. In three patients with herpes simplex and one with herpes zoster,

TABLE 6

THE RELATIONSHIP OF GROUP 1 ADENOVIRUS-INDUCED UPPER RESPIRATORY ILLNESS (URI) TO THE MAXIMUM SERUM TITER TO THE HOMOLOGOUS ADENOVIRUS

	Total	No. With Titer ≥1:128	Signifi- cance
Patients with URI	9	4	$\chi^2 = 2.7$
Patients without URI	7	5	$P > .1$

TABLE 7

COMPARISON OF THE MICROTITER HEMAGGLUTINATION-INHIBITION TEST (HI) TO COMPLEMENT-FIXATION TEST (CF) FOR DIAGNOSTIC CAPABILITY

Fold-Increase CF Titers	Fold-Increase HI Titers*		
	0-2	4	≥8
0-2	8	11	5
≥4	0	0	6

* $\chi^2 = 12.9$; $P < .0005$.

the smears showed inclusion bodies or giant cells, or both. The histology of the other 17 slides was either nonspecific or not diagnostic of viral infection. Few had the typical predominance of mononuclear cell types, and most were composed of a mixed white blood cell population and epithelial cells.¹⁴

Clinical studies—EPIDEMIC KERATOCONJUNCTIVITIS—We observed 56 patients until their clinical symptoms abated and examined them with a slit lamp.⁸ Twenty-eight patients showed signs of keratitis during their illness and had chronic subepithelial infiltrates when examined one month after resolution of their illness (16 of these experienced some degree of decreased visual acuity as well). The 28 remaining patients, who were also infected during the first wave of the epidemic were seen at the same time and had no subepithelial infiltrates. The peak geometric mean hemagglutination-inhibition titer of the former group with residual infiltrates was 1:34.5 (range 1:10 to 1:160). The data for the unaffected group were 1:102.5 geometric mean titer (range 1:20 to 1:320). This difference was significant ($P \leq .001$). (The collection of sera and intervals between examinations were similar for patients because they were part of an epidemic group.³ The serologic tests were exactly the same as those carried out in the nonepidemic group, and often were done simultaneously).^{4,6-8}

NONEPIDEMIC ADENOVIRUS INFECTIONS—Only the patients with Groups 1 and 2 adenovirus infections formed populations of sufficient size and homogeneity for statistical

TABLE 8
CORNEAL INFILTRATES AND ANTIBODY TITERS IN NONEPIDEMIC ADENOVIRUS-INFECTED PATIENTS

Case No.	Adeno-virus Type	Peak Titer*		No. of Eyes	Corneal Involvement		Residual Lesions	Decreased Visual Acuity
		Homologous	Heterologous		Present on 1st Visit	Maximum Involvement† (%) (Worst Eye)		
Group 1								
1	3	128	<4	0	0	0	0	0
2	3	128	<4	2	+	20	0	+
3	7	32	<4	2	0	30	+	0
4	3	128	<4	0	0	0	0	0
5	3	64	5.3	0	0	0	0	0
6	7	128	<4	1	+	20	+	0
7	7	128	6.1	1	+	25	+	0
8	7	16‡	<4	0	0	0	0	0
9	7	64‡	<4	2	0	20	+	0
10	7	128	6.9	1	+	10	0	0
11	3	32	<4	1	+	50	+	+
12	7	128	<4	1	+	30	+	+
13	7	64‡	6.1	1	+	30	0	+
14	7	128‡	4.6	0	0	0	0	0
15	7	256‡	4.6	1	+	20	+	0
16	7	64‡	5.3	2	+	75	+	0
Group 2								
17	9	32	4.2	0	0	0	0	0
18	8	32	4.0	2	+	25	+	+
19	8	32	5.7	2	+	50	+	+
20	8	16	4.0	1	+	35	+	0
21	8	32	11.3	2	0	40	+	0
22	8	32	7.1	2	+	75	+	+
23	8	128	5.7	1	+	100	+	+
24	8	64	8.6	2	+	25	0	+
25	8	64	7.1	2	0	30	+	0
26	8	32‡	5.9	1	+	10	0	0
27	29	64	13.7	2	+	75	+	0
28	8	64‡	4.8	2	+	40	+	0
29	8	32‡	4.0	2	+	40	+	0

* Represents reciprocal of highest observed dilution of antiviral activity to infecting (homologous) virus and the average reciprocal activity against other viruses in that group† (heterologous).

† Estimated maximum percent of cornea with epithelial or subepithelial infiltrates.

‡ Titers did not change significantly ($\geq$ fourfold) during the observation period (compare with Table 5)

analysis of corneal disease. Group 1 was composed of five patients with adenovirus type 3 and 11 patients with adenovirus type 7 infections. Group 2 contained 11 patients with adenovirus type 8 and one each with adenovirus type 9 and adenovirus 29.

The data concerning the corneal involvement and antibody levels of both groups were compiled (Table 8, compare with Table 5). Analysis by χ^2 testing showed several sig-

nificant correlations. First, the number of eyes involved with corneal changes differed significantly in the two groups. In Group 1, there were five patients with no involvement, seven with one eye, and four with both eyes affected. Group 2 had only one patient without corneal changes, three patients with a single eye involved, and nine patients with both eyes involved ($\chi^2 = 5.94$; df, 2; $P \approx .05$). When this same factor

(number of eyes) was used, and the antibody levels were compared for all adenovirus infections (Groups 1 and 2), a more significant difference was noted. At a level of 1:64, there were 12 doubly involved patients, four with one eye, and three with no corneal involvement. At a level of 1:128, only one patient showed infiltrates in both eyes, six had single eye involvement, and three had no ocular involvement. The difference between the number of patients in these three categories proved to be statistically significant ($\chi^2 = 7.65$; df, 2; $P < .025$).

Correlations of the degree of corneal involvement with antibody level were also carried out. There was no difference between the two groups in the number of patients with central lesions vs. peripheral lesions of the cornea. This was reflected in the patients with decreased visual acuity (Table 8).

We devised a comparison of the two groups in terms of fold-rise in antibody titer (Figure). (The fold-rise was a reflection of the rate of antibody production as well as the amount of antibody.) As evidenced by the graph, the responses of the two groups were almost diametrically opposed. For each fold-rise in titer, the Group 1 patients had progressively less corneal involvement. For the same change in titer, the Group 2 patients showed progressively more corneal infiltration. However, the final level of antibody achieved was lower in Group 2 (compare with Table 4).

A similar difference in the two groups was noted at the 40% level of corneal infiltration. Only two patients (of 16) in Group 1 had infiltrates that involved $\geq 40\%$ of the corneal surface area. In Group 2, six of 13 patients were affected in the same way ($\chi^2 = 5.73$; df, 1; $P < .025$).

Finally, age was not a factor differentiating the two groups. The average age of Group 1 patients was 29.9 years; Group 2 patients averaged 30.5 years. The difference was not statistically significant ($t = .128$; df, 25).

HETEROLOGOUS ANTIBODY RISES WITH ADENOVIRUS INFECTIONS (NONEPIDEMIC

Figure (Knopf and Hierholzer). A comparison of the percent corneal involvement with fold-rise in antibody titer among patients infected with Group 1 (AV 1) and 2 (AV 2) adenoviruses. r represents the correlation coefficient of the least-squares line calculated to fit the data. Only patients with at least a fourfold rise are included.

GROUP)—Rises in titers to heterologous viruses were observed in Groups 1 and 2 infections. Thus, in patients infected with adenovirus type 7 (Group 1), a significant change in titer to this agent occurred, as well as smaller changes to such related types as 3, 11, and 21 (Table 8). However, rises in heterologous titers were more consistently seen in patients with Group 2 infections. For example, adenovirus type 8 infections produced rises to 12 related viral types in the Group 2 series. This was reflected in the higher heterologous average titers (Table 8). In particular, we noted a tendency for heterologous responders within family groups. In one of these families, all of the blood relatives showed changes in titer to several other Group 2 subtypes after adenovirus type 8 infection. However, the spouse of one member, exposed to the same virus, had a serologic change to adenovirus type 8 alone (homologous responder).

We observed patients with keratoconjunctivitis from other causes during this study (Table 3), but only adenovirus patients formed homogenous groups that lent themselves to statistical analysis. The herpes group was large (eight herpes simplex, two herpes zoster, two possible mixed herpes simplex-zoster, and three presumed cytomegalovirus), but heterogenous in terms of acute and chronic illness, treatment with various antiviral drugs or corticosteroids, and poor serologic responses. Variability in history, follow-up, and other factors occurred among the patients with adenovirus so the number of patients who met the criteria for various analyses often differed from one test to another. In terms of treatment, however, almost all of the patients with adenovirus required only therapy with topical vasoconstrictors or antibiotics, or both. Only two patients (one each from Groups 1 and 2) required corticosteroids for symptomatic relief, and this was instituted after achieving maximum corneal scores.

Analysis of tears—Ocular external secretions (tears) were analyzed in ten patients. In general, there were low antibody titers. Four patients with serologic diagnoses of herpetic infection represented chronic, recurrent keratoconjunctivitis or kerato-uveitis. Only one patient with disciform keratitis and uveitis had detectable tear-borne neutralizing antibody to herpes simplex. His serum titer, however, was not greater than 1:8 in a simultaneous neutralization test. One other patient showed tear antibody to herpes zoster and serologic changes consistent with simplex. Whether this represented true simultaneous infection or the presence of cross reaction was not determined.

Two patients had high titers (1:8, 1:16) to coronavirus OC-43 in their external secretions. Upper respiratory infection has produced antibody in tears¹¹ and this may represent that kind of relationship.

The predominant immunoglobulin species in the tears was IgA, in agreement with previous work.^{11,12} However, we made no at-

tempt to prove that the measurable hemagglutination-inhibition or serum neutralization antibodies were unique to IgA.

DISCUSSION

Because all patients in the second part of this study were seen during a nonepidemic time period, the cases of keratitis were varied in their etiologies (Table 3). This observation agrees with previous studies that described a similar variety of viral agents responsible for epidemic keratoconjunctivitis keratitis.¹⁵⁻¹⁸

The behavior of the individual viral infections also conformed to expected patterns. Thus, Group 1 adenoviruses caused more upper respiratory and generalized illness, but less keratitis, than Group 2 infections.^{1,2,19-21} Herpes viruses caused more severe keratitis and kerato-uveitis, but clinical illnesses correlated poorly with serologic changes in antibody titer.^{22,23} We also encountered difficulty in culturing any virus after seven to ten days of symptoms. This has been reported before for epidemic keratoconjunctivitis, but it is probably true for many other viral syndromes.^{2,24-26} We, like others, also found the hemagglutination-inhibition test to be superior to complement-fixation as a serum diagnostic procedure in adenovirus infection.¹⁶ No procedure gave consistent results with tears.

There were differences in the extent of corneal involvement observed in Group 1 and 2 infections. The correlations indicate that Group 2 viruses produced a lower level of specific antibody titer (despite a longer time interval between the collection of acute and convalescent sera), and induced more corneal disease in the affected individuals.

These data suggest that the higher antibody titer in Group 1 protected the cornea, supported by examination of the keratoconjunctivitis epidemic of adenovirus type 8, a Group 2 virus. In the latter patients, a range of antibody was observed that was somewhat higher than that of the nonepidemic Group

1. (The reason for this difference is not readily apparent, but does not lie in testing differences, since both sets of sera were examined under identical conditions and often simultaneously.) What is more important, however, is the range of corneal involvement with this single viral agent. Once again, the patients with higher antibody titer experienced less corneal involvement with this usually virulent agent.^{17,19}

The corneal infiltrates may only represent a pattern of infection and the antibody response to it. Certainly more than one viral type can cause this pattern, as shown in the nonepidemic group. Also, the same virus can cause different degrees of involvement (epidemic study), perhaps depending on the level of antibody illicit by the infection.

Since delayed hypersensitivity was not specifically assayed, the elevated humoral antibody in epidemic Group 2 or nonepidemic Group 1 patients may reflect a higher degree of overall immunity, and cellular immunity may be the chief deterrent of corneal disease. However, systemic antibody alone may protect the cornea (and even the fellow eye) from severe involvement. Whether it was humoral or cellular, or both, the nonepidemic and epidemic groups of patients displayed a reasonably similar pattern of protection that correlated with high antibody titer.

We only speculate about the pathogenesis of the subepithelial infiltrates. In studies of immune-complex disease, for instance, rheumatoid arthritis and Aleutian mink disease, antigen-antibody complexes in blood vessel walls can attract complement and inflammatory cells that cause the damage.^{27,28} These complexes are made to persist by maintaining a low titer antibody, and the disease may be completely eliminated by raising the titer.²⁸ The cornea is similar to the collagenous tissue in the walls of blood vessels,²⁹ and these tissues can absorb certain antigens.^{30,31} Therefore, it seems feasible that viral particles might be bound to the stromal collagen, form complexes with low levels of antibody diffusing within the cornea, and thereby act

as a nidus for inflammation. The subsequent fixation of complement and attraction of inflammatory cells completes the subepithelial infiltrate seen by slit-lamp microscopy. Corticosteroids would disrupt the inflammatory response, but it would not remove the complexes. Hence, discontinuation of the drug would allow inflammatory cells to reappear.³² A similar set of circumstances was proposed in the pathogenesis of stromal disease in herpes simplex keratitis.³³

Based on these suppositions, one may conclude that the sometimes permanent visual damage observed in epidemic keratoconjunctivitis might be decreased or eliminated by enhancing the immune response of the infected patient, perhaps during the acute infection.

SUMMARY

Of 120 individuals suffering from follicular conjunctivitis, with or without keratitis, 56 had epidemic keratoconjunctivitis caused by adenovirus type 8. The remaining 64 patients had keratoconjunctivitis produced by several different viruses, including herpes simplex, adenoviruses types 3, 7, 8, 16, 21, and 29, and others. Positive viral cultures were difficult to obtain after the first week of illness in most viral infections. Serologic tests were far more successful in identifying causative agents. Hemagglutination-inhibition tests in adenovirus infections proved to be fast, accurate, and more sensitive than other serologic tests. Among the epidemic and nonepidemic adenovirus groups, the degree of corneal involvement in the infections appeared to be correlated with the level antibody against the infectious agent. Patients with low antibody titers had more severe keratitis than those with high titers. This correlation was true for both the epidemic and nonepidemic patients.

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